REMARKS

I. INTRODUCTION

Claims 31 and 39 have been amended. Claims 1-30 were previously cancelled. Claims 34 and 35 have been cancelled. Claims 41-67 were withdrawn. Thus, claims 31-33 and 36-67 are pending in the present application. No new matter has been added. In view of the above amendments and the following remarks, it is respectfully submitted that claims 31-33 and 36-40 are in condition for allowance. It is noted that the quantitative amendment to claim 31 is being presented to rectify an overlooked typographical error from a translation using a standard in which the use of commas is used to represent decimals while periods are used to represent a break for factors of one thousand. Verification of this translation error may be found by looking at claim 1 of the Certified Copy of Foreign Priority Application.

II. THE RESTRICTION REQUIREMENT SHOULD BE WITHDRAWN

Although the Examiner does not explicitly re-present the restriction requirement, Applicants maintain the position that the claims are of a single general inventive concept under PCT Rule 13.1. Applicants respectfully maintain the previously presented arguments regarding withdrawal of the restriction requirement. In addition, the following responses to the Examiner's bases for finding the argument not persuasive are hereby respectfully submitted.

The Examiner asserts that the common technical feature among the different purported groups is the culture media. The Examiner further states that the intended use of the composition for the expansion of stem cells is not a feature of all the groups. It is respectfully submitted that the previous arguments are not being presented to state that the intended use is the common technical feature among the groups. In fact, it was previously submitted that the culture media was the common feature. The culture media itself is what provides the improved results of using the culture media of the present invention for expanding autologous human stem cells to be realized. The Examiner further stated that in view of Xia, the claimed composition of the present invention is merely an obvious variant and differs only in the concentration of the heparin component. As discussed with regard to the further rejections presented by the Examiner, heparin may be a result effective variable. However, those skilled in the art will understand that this only holds true when concerning the intended use of the media as taught in Xia. A basic

difference between the media of Xia and the present invention is in the intended use of the culture media. Depending on the use of the media, a variety of different components may be a result effective variable.

The Examiner states that the method steps for using a claimed composition is irrelevant unless they require a structural form or feature to be present or absent in the claimed composition. Again, the Examiner resorts to the basis that the media composition of Xia and the present invention are used for the generic autologous human progenitor cells. It is respectfully maintained that the intended use is a further consideration which was not accounted. In addition, in view of the amendments submitted herewith and the explanations below, the structural form is a feature and thus, present in the claimed composition.

III. THE 35 U.S.C. § 112 REJECTION SHOULD BE WITHDRAWN

The Examiner has rejected claims 31-40 under 35 U.S.C. § 112, second paragraph, as being indefinite. (See 3/19/09 Office Action, p. 12).

The Examiner rejects claim 31 for reciting limitations that are unclear as the prior art measures protamine in different concentration units. To expedite processing of the present application, claim 31 has been amended to use concentration units that the prior art also uses as a measure of protamine. Because claims 32-40 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that claim 31 and all depending claims (32-40) are allowance.

IV. THE 35 U.S.C. § 103(a) REJECTION SHOULD BE WITHDRAWN

The Examiner rejects claims 31-37 under 35 U.S.C. § 103(a) as unpatentable over The Journal of Immunology, Xia et al., 2002 (hereinafter "Xia"). (See 3/19/09 Office Action, p. 14).

The Examiner maintains the rejection under Xia providing further explanation. The previously presented arguments are respectfully being maintained. In addition, in view of the amendments being submitted herewith, the culture media and associated methods incorporate a structural aspect as will be described in further detail below.

Claim 31 has been amended to recite an autologous expansion culture medium of autologous human progenitor stem cells comprising "between 0.1% and 90% weight of autologous human serum supplemented with between 0.1 and 10,000 UI/ml heparin and between 0.001 and 100 mg/ml protamine," and "a base culture medium including basic nutrients wherein the autologous human serum is obtained by plasmaphersis with heparin and protamine."

As indicated by the Examiner's responses, it appears that the Examiner assumes that the structure of the serum used in the media described in Xia is the same as the structure of the autologous serum used in the composition of the media described in the present invention. In particular, the Examiner notes that a type of cell to which a media is used is irrelevant to the examination of the composition *unless* they require a structural form or feature to be present or absent. (See 3/19/09 Office Action, p. 4). The Examiner adds that, because plasmapheresis is a well known procedure for obtaining autologous serum, the media composition of claim 31 would be obvious regardless of a potential difference in serum structure. The Examiner argues that the motivation to modify the heparin/protamine concentrations of Xia to those of claim 31 are to conserve resources and lower costs.

It is respectfully submitted that the structure of the autologous serum of the present invention is different from that of Xia. The structure (chemical composition) of the autologous serum obtained by plasmapheresis with heparin/protamine is unlike any obtained by other procedures, including plasmapheresis with Anticoagulant Citrate Dextrose Solution (ACD). One skilled in the art may obtain autologous serum manually, for example, by drawing blood from the patient with a syringe or mechanically by plasmapheresis. The most common way of obtaining autologous serum is the manual drawing of blood from the patient. While the serum isolating procedure is not specified in Xia, those skilled in the art may assume that that the serum was obtained in this manner. Among the instructions for manually drawing blood from the patient and isolating the serum in the drawn blood is allowing the blood to coagulate followed by centrifugation and microfiltration. The coagulation of blood requires the release of coagulation factors from the patelets. These factors remain soluble in the filtered serum and are inevitably present in the culture media used in laboratory research, such as that of Xia. Those skilled in the

art will understand that serum allowed to clot naturally stimulates cell proliferation more than serum from which cells have been removed physically, to which patelet released factors account for this effect. Accordingly, the structural difference between the serum used in Xia and the serum of the present invention is a technical characteristic of the serum used to complement the media that affects cell physiology and accounts for a difference in the outcome of the cell culture exposed to the serum.

In an alternate embodiment, manually drawn blood may be treated with an anticoagulant. Xis explains that in order to isolate monocytes from healthy donors, venous blood is drawn and anticoagulated with a 3.8% sodium citrate solution. Thus, in the event that any assumption regarding the use of anticoagulants for obtaining autologous serum were to be made, it should be that sodium *citrate* is used as the anticoagulant. Autologous serum obtained by drawing blood manually with the use of sodium citrate as the anticoagulant is also structurally different from the serum used in the expansion media of the present invention, as will be explained in further detail below.

In the plasmapheresis procedure, whole blood is continuously removed from the patient through a central venous catheter and enters the pheresis machine through an extracorporeal circuit. Within the machine, cells are immediately separated from plasma by filtration, suspended in replacement fluid and promptly returned to the subject's body, while plasma is put to its desired use. Despite no time being allowed for clotting, using an anticoagulant is highly recommended in plasmapheresis to allow the flow of blood from the patient's body to the pheresis machine, where commonly used anticoagulants include heparin and ACD. Once the plasmapheresis procedure is finished, protamine is given to the patient in order to neutralize heparin and allow for the normal physiology of reconstructed blood. A neutralizing dose of protamine will be added to the acellular filtered plasma. Then, plasma proteins will be allowed to clot, due to the presence of calcium, and cleared plasma retrieved by centrifugation. It is noted that acellular plasma protein coagulation does not involve the releasing of coagulating factors. This structural difference allows an optimal physiological environment for the cells being expanded for autologous drafting purposes.

Those skilled in the art will understand the biochemical basis for the anticoagulating action of ACD being the chelating of calcium by citrate. Acellular clotting of proteins will not occur in the absence of calcium, therefore the plasma obtained by plasmaphareris with ACD is structurally different from the plasma obtained by plasmapheresis with heparin/protamine because it contains additional proteins or increased concentration of proteins that may affect the fate of cells being expanded for autologous drafting purposes. For example, such proteins may act as adyuvants in the immune response to transplant rejection. Moreover, calcium is a signaling molecule for cell attachment, cell growth, and differentiation. The centrally positioned signaling molecule Ras is very sensitive to calcium levels. This small GTPase operates as a binary molecular switch and regulates cell proliferation and differentiation. A cell in culture decodes a variety of $InsP_3$-dependent Ca^{2+} signals in time, amplitude, and space during the process of cardiac cell differentiation and heart development. Studies performed in embryonic stem cell differentiating in cardiomyocytes have uncovered that Ca^{2+} regulates multiple steps of cell differentiation. These include secretion of cardiogenic factors, cardiac transcriptional cascades and in turn gene expression, myofibrillogenesis, and initiation of embryonic pacemaker activity. Thus, Ca^{2+} is a major second messenger directing the fate of stem cells. Its absence, due to the presence of chelating factors like citrate, undoubtedly affects the differentiation of progenitor stem cells and their gene expression which may lead to immune rejection upon transplantation.

Thus, it is respectfully submitted that the structure of the autologous serum specified in the expansion media as recited in claim 31 confers a special technical feature to the expansion media such that it results optimally for expanding progenitor-stem cells for autologous drafting purposes. Accordingly, at the time the present application was filed, the media of the present application constitutes an alternative media suitable for expanding progenitor stem cells, in particular muscle progenitor stem cells.

It is also respectfully requested that the inventive step be acknowledged in spite of plasmapheresis being a well established procedure for obtaining autologous serum in view of the recitation of claim 31 because plasmapheresis is not an object of the present invention. That is,

the object of claim 31 is an expansion media composition comprising autologous serum obtained by plasmapheresis with heparin/protamine among other technical characteristics recited therein.

Furthermore, due to the translation error that has been corrected in the present Amendment for the 3/19/09 Office Action, it is respectfully submitted that the motivation indicated by the Examiner is in fact opposite to what the present invention would reasonably provide. As amended, the heparin concentration is between 25 U/ml and 10,000 U/ml. Thus, this would not lead one skilled in the art to reasonably conclude that resources are conserved nor lower costs. Instead, the costs would increase as the concentration would require about 400 times the amount of heparin.

For at least the above described reasons, it is respectfully submitted that Xia does not disclose or suggest the recitation of claim 31. Accordingly, it is respectfully submitted that claim 31 is allowable and the Examiner should withdraw the 35 U.S.C. § 103(a) rejection for this claim. Because claims 32, 33, 36, and 37 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that these claims are also allowable.

The Examiner rejects claims 31-40 under 35 U.S.C. § 103(a) as unpatentable over U.S. Pat. Pub. No. 2002/0124855 to Chachques in view of U.S. Pat. No. 7,015,037 to Furcht et al. (hereinafter "Furcht") in further view of U.S. Pat. No. 4,735,726 to Duggins in further view of U.S. Pat. No. 6,624,141 to Yang et al. (hereinafter "Yang"). (See 3/19/09 Office Action, p. 17).

The Examiner states that the media disclosed in Chachques could be added autologous serum in view of Furcht. The Examiner bases the motivation to choose the autologous serum from the list of suitable alternatives from the fact that Chacques emphases the importance of avoiding an immune response by using autologous cells combined with the fact that Furcht teaches that the same cells can be cultured with autologous serum as well as fetal calf serum. (See 3/19/09 Office Action, pp. 9-10). However, the previously presented arguments are respectfully being maintained. Furcht explicitly addresses the solutions for preventing immune rejection by disclosing specific approaches for transplantation to prevent immune rejection. (See Furcht, col. 28, l. 61 – col. 29, l. 25). In this discussion, Furcht does not include the use of

autologous serum. Due to the detail involved in the description of this section, one skilled in the art would reasonably assume that autologous serum would have no effect on the outcome of cells expanding for drafting purposes in view of the teachings of Furcht. Thus, the likelihood of adding autologous serum to the media is not a reasonable combination between Chacques and Furcht. Furthermore, despite using plasmapheresis with heparin/protamine, the medium of the present application is not necessarily obvious. Despite one in the art potentially reaching the solution in order to provide for an alternative culture media that diminishes rejection upon transplantation (which is not conceded), the skilled person in the art would not have decided to combine the teachings in the cited documents as a solution to the problem of avoiding rejection.

Thus, for the reasons discussed above, it is respectfully submitted that the cited references do not obviate the recitation of claim 31. Accordingly, it is respectfully submitted that claim 31 is allowable and the Examiner should withdraw the 35 U.S.C. § 103(a) rejection for this claim. Because claims 32, 33, and 36-40 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that these claims are also allowable.

The Examiner rejects claims 31-40 under 35 U.S.C. § 103(a) as unpatentable over U.S. Pat. No. 6,472,212 to Valerio et al. (hereinafter "Valerio") in view of U.S. Pat. No. 5,817,773 to Wilson et al. (hereinafter "Wilson"). (See 3/19/09 Office Action, p. 19).

The Examiner states that Valerio discloses culture media compositions comprising basic media, autologous human serum, antibiotics, and protamine sulphate. As discussed previously, this media is suitable for culturing bone marrow cells. Those skilled in the art will understand that in the field that bone marrow cells and, in general, cells of the blood lineage, do not need attachment to substrates in order to be expanded. That is, these cells grow in *suspension*. This is an important property or technical character that is taken into account when designing media composition because culture media designed for suspension-growing cells may not support attachment dependent growing cells.

Furthermore, as indicated by the Examiner as well, cells are not part of the composition of the claimed media. The media is suitable for expanding progenitor stem cells, as recited in

claim 31. This limitation is a technical characteristic of the present invention. Those skilled in the art will understand that progenitor stem cells are attachment-dependent. Culture media that does not allow attachment of cells or permits only poor attachment of cultured cells to the plate surface will result in a high rate of detachment and death of expanding progenitor stem cells, thereby precluding expansion to cell counts suitable for transplantation. The claimed media composition presents high autologous calcium content which is critical for cell attachment. The presence of autologous calcium in the media is a consequence of the novel combination of media components as that recited in claim 31. Specifically, as described above, the autologous serum chosen by its isolation method (*i.e.*, plasmapheresis with heparin/protamine) results in high levels of autologous calcium in the final media composition.

Furthermore, the previous presented arguments regarding the above cited references are respectfully being maintained. It is respectfully noted that it appears that the Examiner is constructing the claimed media by adding unconnected references solely on the basis that they contain the element that is suitable to obtain the claimed media regardless of the functionality that component may provide the respective reference. It is respectfully submitted that the functionality of the component is a critical factor that is part of the culture media and that the Examiner's attempt at randomly selecting references to obviate claim 31 is impermissible hindsight. It is also respectfully submitted that attention should be drawn to the fact that the final media composition is not a simple addition of the parts contained therein but a consequence of the physiological advantages resulting from the orderly addition of these components. It is not reasonable to add the histamine to the protamine-containing medium of Valerio because the medium was designed to have a particular physiological effect on the growing cells. This physiological effect is a technical characteristic of the media and it is provided by its protamine content. Those skilled in the art would understand that a selection of a starting media with technical characteristics that are suitable for solving the problem is critical in attempting to find a final media. To subsequently neutralize the technical characteristic that was specifically selected for the trials would run contrary to basic experimentation principles. That is, it is respectfully requested that the Examiner note that to negate a technical characteristic of an already existing component that was specifically chosen would be unreasonable and illogical for one skilled in the art.

In addition, it is respectfully submitted that to properly obtain the final media with the expected characteristics that are desired, the order in which the components of the media are added is critical for obtaining the specific properties of the claimed media. The specific characteristics are technical in character and specifically make the media suitable for expanding progenitor stem cells.

Thus, for at least the above reasons, it is respectfully submitted that claim 31 is allowable and the Examiner should withdraw the 35 U.S.C. § 103(a) rejection for this claim. Because claims 32, 33, and 36-40 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that these claims are also allowable.

CONCLUSION

In light of the foregoing, Applicants respectfully submit that all of the now pending claims are in condition for allowance. All issues raised by the Examiner having been addressed, and an early and favorable action on the merits is earnestly solicited.

Respectfully submitted,

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